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Lovastatin inhibits visceral allodynia and increased colonic permeability induced by lipopolysaccharide or repeated water avoidance stress in rats

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ABSTRACT

Statins have been reported to block inflammatory somatic pain and have an anti-cytokine property. Lipopolysaccharide (LPS) or repeated water avoidance stress (WAS) induces visceral hypersensitivity and increases gut permeability in rats, which are mediated through proinflammatory cytokine-dependent pathways. Since visceral hypersensitivity with increased gut permeability plays a crucial role in the pathophysiology of irritable bowel syndrome (IBS), these above animal models are considered to simulate IBS. We hypothesized that lovastatin improves symptoms in the patients with IBS by attenuating these visceral changes. The threshold of visceromotor response (VMR) induced by colonic balloon distention was measured for the assessment of visceral sensation in rats. Colonic permeability was determined in vivo by quantifying the absorbed Evans blue in colonic tissue for 15 min using a spectrophotometer. Subcutaneously (s.c.) injected LPS (1 mg/kg) reduced the threshold of VMR after 3 h. Pretreatment with lovastatin (20 mg/kg s.c. daily for 3 days) abolished this response by LPS. Repeated WAS (1 h daily for 3 days) induced visceral allodynia, which was also blocked by repeated injection of lovastatin before each stress session. The antinociceptive effect of lovastatin on the LPS-induced allodynia was reversed by mevalonolactone, N^G-nitro-L-arginine methyl ester or naloxone. Lovastatin also blocked the LPS- or repeated WAS-induced increased gut permeability. These results indicate the possibility that lovastatin can be useful for treating IBS.

1. Introduction

Disturbed gut motility and altered visceral sensory function are considered to play an important role in the pathophysiology of irritable bowel syndrome (IBS) (Taché et al., 2009). Additionally, the importance of immune system activation has been also indicated (Bercik et al., 2005; Elsenbruch, 2011). There is evidence that increased levels of plasma proinflammatory cytokines and serum lipopolysaccharide (LPS) together with enhanced gut permeability are observed in IBS (Dlugosz et al., 2015; Ortiz-Lucas et al., 2010; Sinagra et al., 2016; Zhou and Verne, 2011). Moreover, LPS-induced stimulation of cytokines release from peripheral blood mononuclear cells is enhanced, and higher symptoms severity such as urgency, diarrhea, etc. are associated with higher cytokines response induced by LPS (Liebregts et al., 2007).

We previously showed that LPS induced visceral allodynia via interleukin (IL)-1 and IL-6 pathways (Nozu et al., 2017b). Furthermore, repeated water avoidance stress (WAS)-induced visceral allodynia, which is considered to be an experimental animal model for IBS (Larauche et al., 2012), was also mediated via IL-1 and IL-6 pathways, similar to LPS (Nozu et al., 2017c). In this context, LPS-cytokine system is considered to be associated with the altered gastrointestinal functions in IBS, and anti-inflammatory therapy by inhibiting LPS-cytokine signaling may be a promising approach for the treatment of this disease.

Statins inhibit the enzyme 3-hydroxyl-3-methylglutaryl coenzyme A (HMG-CoA) reductase (Grundy, 1988), and reduce blood cholesterol level, leading to the prevention of cardiovascular diseases (Kazi et al., 2017). However, the risk reduction of these diseases is observed even in the absence of a significant decrease of cholesterol level (Oesterle et al., 2017), and pleiotropic effects of statins such as inhibition of monocyte activation, the production of inflammatory cytokines, etc. (Inoue et al.,

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2000; Methe et al., 2005) could be involved with this phenomenon (Oesterle et al., 2017).

Besides, anti-inflammatory and anti-cytokine actions by statins are also showed in the various animal models, such as inflammatory arthritis (Leung et al., 2003), carrageenan-induced paw edema (Goncalves et al., 2011), among others, and the drugs are also known to suppress cytokine production in intestinal intraepithelial lymphocytes (Zhang et al., 2013) and exhibit antinociceptive action in several animal pain models (Garcia et al., 2011; Santodomingo-Garzon et al., 2006).

Therefore, we hypothesized that statins are beneficial for the treatment of IBS by attenuating visceral hypersensitivity through the anti-cytokine action. In this study, in order to examine the hypothesis, we attempted to determine the effects of lovastatin on visceral allodynia and increased gut permeability induced by LPS or repeated WAS in rats.

2. Materials and methods

2.1. Animals

Adult male Sprague Dawley rats (Charles River Laboratory, Atsugi, Japan) weighing approximately 300 g were used. The animals were housed in groups (3–4 rats/cage) under controlled conditions of illumination (12-h light/dark cycle starting at 7 a.m.), and temperature was regulated at 23–25 °C with food (Solid rat chow, Oriental Yeast, Tokyo, Japan) and water available ad libitum.

2.2. Chemicals

LPS obtained from Escherichia coli with the serotype 055:B5 (Sigma-Aldrich, St. Louis, MO, USA); naloxone hydrochloride, an opioid receptor antagonist; N^G-nitro-L-arginine methyl ester (L-NAME), a nitric oxide (NO) synthesis inhibitor (Wako Pure Chemical Industries, Osaka, Japan) and mevalonolactone (Tokyo Chemical Industry, Tokyo, Japan) were dissolved in normal saline. Lovastatin (Tokyo Chemical Industry) was dissolved in dimethyl sulfoxide (Sigma-Aldrich). The chemical doses were determined according to previous studies (Mirhadi, 2011; Nozu et al., 2017a, 2017b).

2.3. Measuring visceral sensation

Visceral sensation was assessed by colonic distention-induced abdominal muscle contractions (visceromotor response; VMR) using electromyogram (EMG) in conscious rats (Ness and Gebhart, 1988; Nozu et al., 2017b, 2017c).

2.3.1. Implanting electrodes and placing colonic distention balloon

Under brief ether anesthesia, the electrodes (Teflon-coated stainless steel, 0.05-mm diameter, MT Giken, Tokyo, Japan) were inserted approximately 2 mm into the left external oblique musculature though a small skin incision. They were fixed to the musculature by cyanoacrylate instant adhesive together with the incised skin, and the electrode leads were directly externalized through this closed incision. A distension balloon (6-Fr disposable silicon balloon urethral catheter, JU-SB0601; Terumo Corporation, Tokyo, Japan) was intra-anally inserted, with the distal end positioned 2 cm proximal to the anus.

2.3.2. Colonic distention and measuring abdominal muscle contractions

After completing electrode implantation and balloon placement, the rats were placed in Bollmann cages and acclimated to experimental conditions for 30 min before measuring. The electrode leads were then connected to an EMG amplifier, and EMG signals were digitized by a PowerLab system (AD Instruments, Colorado Springs, CO, USA) and recorded by a computer software (LabChart 7; AD Instruments). Colonic distension was performed at 30 min after the surgery, as previously described (Nozu et al., 2017b, 2017c). Namely, the ascending method of limits paradigm with phasic distensions was applied by manually inflating the balloon with water using a syringe, and the distention increased progressively in 0.1 ml steps for 5 s until significant sustained abdominal muscle contractions, i.e., VMR, were detected (Fig. 1A). The VMR threshold was defined as the distended balloon volume (ml) inducing VMR. The threshold was measured twice (2-min interval), and the threshold mean was calculated as the data of the animals. The percentage change threshold, i.e., the threshold value after drug administration divided by the basal threshold value and multiplied by 100, was also calculated.

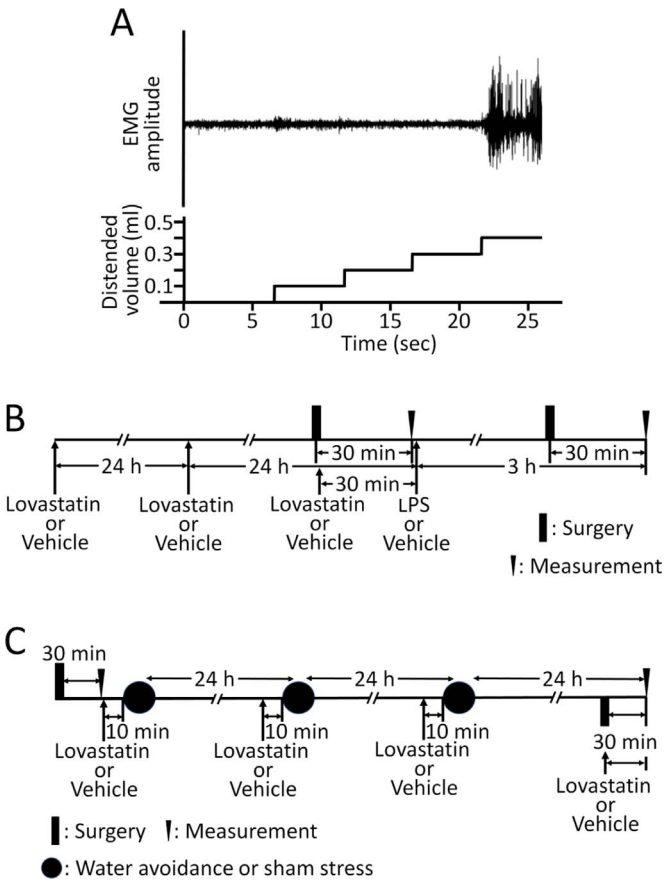

Fig. 1. A The threshold of visceromotor response (VMR) was determined by the distended balloon volume (ml) inducing apparent sustained abdominal muscle contractions. Demonstrable EMG recording is depicted. The threshold was 0.4 ml in this animal. **B** Schematic representation of the experimental protocol. The basal VMR threshold was measured at 30 min after the surgery for implanting EMG electrodes and placing the balloon, and LPS (1 mg/kg) or the vehicle was administered. Later, the surgery and balloon placement were performed again, and the threshold was measured at 3 h after the injection. Lovastatin (5, 20 or 50 mg/kg) or the vehicle was injected thrice at 48 h, 24 h and 30 min before injection of LPS. **C** The basal threshold was measured, and the rats were subjected to either water avoidance or sham stress for 1 h daily for 3 consecutive days. The second threshold measurement was performed at 24 h after the last stress session. Lovastatin or the vehicle was injected 4 times, i.e., at 10 min before each stress session and 30 min before the second measurement.

2.3.3. Experimental procedures

First, the basal VMR threshold was measured. The electrodes and distention balloon were then removed, and either the vehicle or LPS at a 1-mg/kg dose was subcutaneously (s.c.) injected. The rats were returned to their home cages, and after 2.5 h, they underwent surgery for electrode implantation and balloon placement again. The second measurement of threshold was performed 3 h after the injection. The vehicle or lovastatin (5, 20 or 50 mg/kg) was s.c. injected thrice at 48 h, 24 h and 30 min before injecting LPS or the vehicle (Fig. 1B).